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INTEGRATION OF DESIGN OF EXPERIMENT, SURFACE RESPONSE METHODOLOGY AND MULTI-LAYER VALIDATION TO PREDICT THE EFFECT OF BLANCHING ON COLOUR OF TOMATO JUICE

Running title : A model to predict colour change of tomato juice

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ABSTRACT

Response surface methodology was used to develop models to predict the effect of tomato cultivar, juice pH, blanching temperature and time on colour change of tomato juice after blanching. The juice from three tomato cultivars with adjusted pH levels ranging from 3.9 to 4.6 were blanched at temperatures from 60-100 °C for 1-5 min using the central composite design (CCD). The colour change was assessed by calculating the redness (a/b) and total colour change (ΔE) after measuring the Hunter L, a and b values. Developed models for both redness and ΔE were significant ($p<0.0001$) with satisfactory coefficient of determination (R^2 = 0.99 and 0.97) and low coefficient of variation (CV% = 1.89 and 7.23), respectively. Multilevel validation that was implemented revealed that the variation between the predicted and experimental values obtained for redness and ΔE were within the acceptable error range of 7.3 and 22.4%, respectively.

Keywords: tomato, colour, blanching, response surface methodology, modelling

PRACTICAL APPLICATIONS

Thermal blanching is an essential pre-treatment carried out extensively in tomato juice processing industry. Scientific evidence and experience show that fruit variety or cultivar and the exact blanching conditions can affect either positively or negatively the tomato juice colour, the most important quality attribute of the product. Therefore, optimisation of blanching conditions as performed using the current thermal technology in order to obtain desired colour properties is still very important. In this study the impact of tomato cultivar, juice pH and blanching conditions (temperature and time) on the colour of tomato juice after blanching was evaluated and models have been developed that predict the redness and colour change after processing. The models were incorporated into an access web tool we developed that could be beneficial in optimising the processing conditions for a particular tomato cultivar in an industrial environment.

INTRODUCTION

Colour of fruits and vegetables is attributed to phytochemicals known as pigments or bio-chromes. The main pigments are chlorophyll, carotenoids, anthocyanin and betalains. Among them, the family of carotenoids is the most abundant group. They are responsible for many of the red, orange and yellow coloured fruits and vegetables (Delgado-Vargas *et al*. 2000). Tomato, in particular, is one of the main economically important vegetables consumed throughout the world. Colour preservation after processing and during storage has been a major challenge to the tomato juice industry. The stability of carotenoids, especially lycopene – the main carotenoid - during processing and storage is crucial as degradation can lead to loss of product attractiveness and acceptability. The common degradation pathways are isomerisation, oxidation and fragmentation of lycopene, promoted by heat and light (Lin and Chen 2005). Oxidation is a complex process and depends on many factors, such as processing conditions like pH, moisture, temperature, time and the presence of pro- or anti-oxidants and lipids (Shi and Le Maguer 2000). The amount of sugar, organic and amino acids also affects the colour of processed tomato products by causing the formation of 5-hydroxymethyl furfural and other decomposed brown coloured products due to the Maillard reaction (Gould 1992). Pigment degradation is correlated with colour, measured as L, a, b values of the Hunter system expressing the 'brightness', the 'green-red' and the 'blue-yellow' axis, respectively (Tijskens *et al*. 2001).

Hot break (80-100 °C /1-2 min) and cold break (below 65 °C/1-2 min) treatments are commonly applied blanching techniques in commercial tomato juice processing to inactivate enzymes that produce off-flavours and release intracellular gases of the plant tissue, rather than inactivating pathogens and other microorganisms (Hayes *et al.* 1998). In addition, blanching helps in preserving the colour of tomato juice by inactivating enzymes responsible for enzymatic

browning and also enhancing the colour by improving the bioavailability of lycopene since it breaks down the cellulose structure of the plant cell (Stahl and Sies 1992). However, thermal treatment is also responsible for the degradation of red coloured lycopene pigments present in tomatoes by non-enzymatic browning (Maillard reaction) at severe processing conditions (Shi and Le Maguer 2000; Jayathunge *et al*. 2015). Therefore, understanding of the influence of different tomato cultivars and processing factors (such as the juice pH, blanching temperature, time, and their interrelations) on colour change of tomato juice is essential.

Response surface methodology (RSM) with or without central composite design (CCD) has been successfully used for improving and optimising processes by establishing the relationship of processing variables and output variables in many fields (Tiwari *et al*. 2008; Patras *et al*. 2009; Rawson *et al.* 2010; Graham-Acquaah *et al.* 2012). The advantages are: i) reduction in the number of experimental trials needed to evaluate multiple parameters, and ii) the ability of the statistical tool to identify interactions among different variables and provide a mathematical model using appropriate experimental design, as an opposed to traditional 'one-variable-at-a-time' analysis (Myers and Montomery 2002). In the literature, the use of RSM to model colour change of tomato juice is very limited; a few studies have been conducted using non thermal processing technologies and relatively small datasets. Processing conditions in relation to colour of tomato juice have been explored with RSM after high pressure processing (Porretta *et al.* 1995), high intensity pulsed electric fields processing (Aguilo-Aguayo *et al.* 2009) and sonication (Adekunte *et al*. 2010). However, these technologies have not been adopted in the tomato processing industry; thermal treatments such as pasteurisation/sterilisation treatment are still used extensively. Therefore, the application of RSM to optimise the effect of industrial thermal treatment on colour is unexplored. In particular, the impact of blanching conditions on

colour change of tomato juice in relation to all the main processing parameters, i.e. juice pH, blanching temperature and time, remains unknown. Development of such a model to predict the colour change of tomato juice during thermal blanching will be beneficial for the tomato juice processing industry to enable them to decide the optimum blanching conditions in order to achieve the desirable product colour after blanching.

Therefore, the aim of this study was to develop and rigorously validate a practical RSM-based model to predict the colour (redness and total colour change [ΔE]) of tomato juice during blanching conditions (temperature and time) at different juice pH levels for three different cultivars using a large data set. Although the variety of tomato has a major impact on colour of tomato juice, this is the first study that looks at a number of common cultivars and makes it the optional parameter in the model design.

MATERIALS AND METHODS

Fruit and other materials

Ripe tomatoes of three cultivars, namely ‘Baby plum’, ‘Truss’ (medium vine) and ‘Loose’, were purchased on 11 different occasions (3 in the summer, 2 in the winter, 3 in the autumn and 3 in spring season) from local stores in Northern Ireland (UK) between January and December 2014. In total, 50 kg of tomatoes were purchased and processed during this period. The origins of each tomato cultivar were as follows: ‘baby plum’ - Spain (5), Canary Islands (3), Morocco (1), Senegal (2), France (2); ‘Truss’ -Spain (6), Netherlands (5); and ‘Loose’ -Spain (5), Netherlands (4). All tomatoes were graded before processing, odd shaped and sized fruits were excluded.

Cheese cloth (100 × 100 cm) was purchased in the local market and standard packaging materials (polyethylene/polyamide film) were obtained from Andrew James (Durham, UK).

Sample preparation

Tomatoes were washed, cut into pieces (6x6 cm) and crushed using a household blender. The seeds and skin of the tomato were removed by passing the juice through a cheese cloth. The resulting juices from each cultivar were subjected to pH adjustments to 3.9, 4.26 and 4.6 by adding citric acid or NaOH (0.1 M) and pH was monitored using a pH meter (JENWAY 3510, Staffordshire, UK). Samples of tomato juice (50 ml, total soluble solid content- 5 °Brix) were transferred into heat sealed polyethylene/polyamide pouches (15x10 cm) and kept for a short period of time (15 min) under refrigeration (4 °C) before blanching.

Blanching

Tomato juice samples were blanched using a Grant GD100 water bath (Grant Instruments, UK) at three different blanching temperatures (60, 80 and 100 °C) for three time periods (1, 3 and 5 min) according to the experimental design (Table 1). Blanching conditions (temperature and time) implemented in this study was selected based on the hot break (80-100 °C) and cold break (below 65 °C) methods applied in the industry (Hayes *et al.* 1998). The temperature of the juice during blanching was monitored using a digital thermometer (HI 98804, Hanna Instruments, UK) fitted with a k-type thermocouple, and samples were cooled in iced water immediately after blanching.

Colour measurements

Colour of the blanched and fresh tomato juices were measured using a Minolta CR-410 portable colourimeter (Konica Minolta, Japan). A standard white tile (X= 87.01, Y=0.3185, Z=0.3365) was used to calibrate the instrument. The juice samples were placed in a glass Petri dish on top of the light source and L, a, b values were directly taken from the colourimeter. Each measurement reported represents the average of three readings. L, +a, -a, +b, –b represent lightness, redness, greenness, yellowness and blueness, respectively. The Hue value (a/b) and the total colour change ($\Delta E=\sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$) were calculated based on measured L, a and b values.

Experimental design and statistical analysis

A central composite design (CCD) for four factors (tomato cultivar, pH, blanching temperature and time) at three levels (lower, middle and upper, coded as -1, 0 and +1) was used to study the response on the colour changes (Table 1). Inputting all the parameters to Design Expert 7 software (Stat-Erase, Inc., Minneapolis, USA), a total of 30 runs were required in the design in order to create the model (as opposed to the 81 in the original design). The experiment was replicated using tomato cultivars produced in different countries in order to capture seasonal and compositional variability (see 2.1) and data were fitted to the following second order polynomial model:

$$Y = \beta o + \sum_i \beta iXi + \sum_i \beta iiXi^2 + \sum_{ij} \beta ijXiXj \qquad (1)$$

Where: Y is the predicted response, β_0 the constant (intercept), βi the linear coefficient, βii the quadratic coefficient and βij is the cross product coefficient. Xi and Xj are independent variables.

To perform this operation, the same software was utilised to analyse the statistical characteristics of the data and to develop a prediction equation between process variables and response colour

changes. According to the experimental data, nine different fitting equations for each factor represented by Equation 1 were constructed and the overall equation obtained by calculating the average of each model term coefficient. This was considered the final model (Fig. 1). The statistical significance of the model terms was examined by ANOVA ($p<0.05$). The adequacy of the model was determined by evaluating the lack of fit, coefficient of regression (R^2) and the Fisher test value (F value). Statistical significance of the model and model variables were determined at the 5% probability level ($p<0.05$) and three dimensional response surfaces were built using the software according to the model equation shown in above.

Evaluation of calibration data performance using cross validation

In order to evaluate the accuracy of the calibration data used in the model development 'leave-one-out' cross validation was employed. Nine different fitting equations obtained in the section 2.5 were used in performing the 'leave-one-out' cross validation method. Whole experimental data were divided into 9 parts based on the day and each 1/9th in turn (30 data points) was removed. A model was built on the $8/9^{th}$ data left in and the left out data were predicted from the new model. This was repeated with each 1/9th of the data until all the data (270 data points) had been predicted and considered as nine iterations. The average prediction error for both redness and ΔE, in each iteration sets was calculated.

Model validation and performance evaluation

The predictive performance of the model was validated within the design space. A random set of 60 experimental combinations was used in independent experiments (2×30) and conducted at the fifth and twelfth months of the experimental period (Fig. 1). A random set of 60 combinations consisted of both exact and in-between points of processing parameters. The experimental and

predicted values were compared in order to determine the validity of the model. Moreover, the model performance was assessed using standard criteria of accuracy factor (AF, Equation 2), biased factor (BF, Equation 3) and average mean deviation (E%, Equation 4) (Baranyi *et al.* 1996). Equations AF and BF explain the prediction ability or how well a model can predict the new data. The value of AF and BF should be close to 1, if a model is optimal. E % describes the variation between expected and predicted which should be as close to zero as possible.

$$AF = 10^{\frac{\sum|(\log Vp/Ve)|}{Ne}} \qquad (2)$$

$$BF = 10^{\frac{\sum(\log Vp/Ve)}{Ne}} \qquad (3)$$

$$E\ (\%) = \frac{1}{Ne}\sum|(Ve - Vp)/Ve)| \times 100 \qquad (4)$$

Where: N_e is the number of experimental data, Ve is the experimental value and Vp is the predicted value.

The flow chart representing the overall procedure conducted to calibrate and validate the predictive model is given in Fig. 1.

RESULTS AND DISCUSSION

Selection of design variables

In general, maintenance of the bright red colour of tomato juice during processing is influenced by several inherent factors such as cultivar and processing factors such as juice pH, processing temperature, time. Their effects may be either independent or interactive (combined effect of one factor with another factor). Hence, these exact parameters were selected as the input variables in

this experimental design and the levels of each variable were selected to simulate the conditions applied in industrial tomato juice processing. Selecting the right output variables was essential because the dominant colour of tomato juice is a mixture of red and yellow. Logically, Hunter a and b value, or some combination of a and b, should be considered as the physical parameters to describe the visual colour change of tomato juice. The ratio of a/b, also known as redness, related to the hue angle, has been used to describe and model the colour changes of tomatoes (Thai *et al*. 1990). Due to the association of a and b with the L value (Ahmed *et al*. 2002), representation of colour as a function of L, a and b values is required. Hence, apart from redness (a/b), the total colour change ($\Delta E=\sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$) was also selected to represent tomato juice colour change after blanching treatment.

Model development and fitting

The experimental data were used to calculate the coefficients for the linear, interaction and quadratic factors, which were used to predict the responses of redness and ΔE after blanching and presented in Table 2.

Analysis of variance (ANOVA) was used to evaluate the significance of the model as well as the linear, quadratic and interactive influence of input variables on each reponses (Table 3). Results revealed that the developed models are highly siginificant ($p<0.0001$), i.e. the model parameters have significant influence on the responses (redness and ΔE). Moreover, the predictive variables have non-linear influences on the responses of redness and ΔE since the quadratic and interactive terms were also significant at $p<0.05$. The coefficient of regression (R^2) is the proportion of variability in the data explained or accounted by the model. Le *et al*. (2010) and Chauhan and Gupta (2004) have emphasised the acceptance of any model with $R^2>0.75$. A high proportion of

variability was explained by the RSM models for redness and ΔE, as indicated by high regression coefficients (R^2 = 0.99 and 0.97) and low coefficient of variation (CV = 1.89 and 7.23%), respectively. This model exhibited an insignificant lack of fit ($p>0.05$), indicating the high applicability of the model.

In general, the colour of tomato juice is influenced by culivar, pH, blanching temperature and holding time. ANOVA showed that the most influential factor for redness ($F=952.21$, $p<0.0001$) and ΔE ($F=310.94$, $p<0.0001$) of tomato juice after blanching was the cultivar. Blanching temperature also exhibited a significant ($p<0.0001$) influence on the redness and ΔE values, whereas the rest of linear effects, juice pH and blanching time, showed non-significant (p values 0.2611 and 0.3333, respectively) influence on tomato juice after blanching. Regarding the interactive effects, a significant contribution was between cultivar and temperature ($F=38.34$, $p<0.0001$) and between pH and temperature ($F=11.17$, $p=0.0045$) for the ΔE, while quadratic effects of cultivar ($F=111.32$, $p<0.0001$) and blanching temperature ($F=18.21$, $p=0.0007$) showed significant influence on redness of tomato juice after blanching. In this study, blanching time exhibited negative coefficients for redness whereas juice pH and blanching temperature presented negative coefficients for the ΔE, indicating that higher levels of those factors produced lower redness and ΔE respect to the fresh tomato juice.

Analysis of response surfaces contours

The fresh juice instrumental colour data (L, a, b and redness [a/b]) was fairly consistent over the entire sets of experiments for the period of 12 months, with small standard deviation from the mean (data not shown). The redness values were 1.48, 1.70 and 1.27, for cultivars 1 (baby plum), 2 (truss) and 3 (loose), respectively. The observed differences in colour between cultivars could

be attributed to inherent factors and growing conditions of these cultivars as well as to the different geographical locations in which they were grown (Gould, 1992).

The relationship between independent and dependant variables is illustrated in two dimensional representation of the response surfaces contour plots (Fig. 2 and 3). Tomato cultivar has a great influence on the redness and ΔE after blanching; therefore it is the processor's responsibility to select the suitable cultivar for tomato juice processing. Results indicate that cultivar 1 and 2 had higher redness values due to the original juice colour at any particular time, the best redness was obtained when the temperature was between 60-80 °C, above which colour was degraded. This is consistent with the fact that temperature and cultivar factors were significant influencing factors. Tomato lycopene, which is responsible for the tomato's bright red colour, can be released during heat treatments through disruption of cell matrix making them more extractable (Perez-conesa *et al*. 2009). However, severe temperature conditions, resulted in brownish colour products with lower instrumental values of redness (a/b). The same results can also be made for ΔE and it was observed to be very distinct at higher temperature conditions. These colour alterations may be explained by carotenoid degradation due to heat, as stated by Barreiro *et al.* (1997) for double concentrated tomato paste. On the other hand, low temperature and short time treatment also gives lower redness and ΔE, as shown in Fig. 2f and 3f; indicating that this combination was not sufficient for effective lycopene extraction in tomato juice.

It is also clear from Fig. 2e and 3e that ΔE of tomato juice was independent of the tested pH range of 3.9 to 4.6; this might be due to the tested pH range not being adequate to significantly influence the colour qualities. This finding contrasts with Porretta *et al.* (1995), who reported higher redness (a/b) of tomato juice when high pressure processed at pH 4.5 in comparison to juice at pH 4 and 5. The combined effect of pH and temperature on the ΔE of tomato juice

revealed that ΔE was minimal at low temperatures irrespective of the juice pH. Similarly, the time limits (1-5 min) were not effective to obtain significant impact on the colour qualities as revealed by the results.

Calibration data performances

The prediction error calculated for redness and ΔE for each iteration set analysed by the method of cross validation was calculated. The prediction error for redness varied 5.91-18.67 % whereas the range for ΔE was 12.60-33.40 % (Supporting material 1). The prediction error for redness remained lower in all iterations in comparison to ΔE, except the iteration number 1. The average prediction error for redness and ΔE was denoted as 10.25 and 20.57%, respectively. Hence, the minimum prediction error of 10.25 and 20.57%, respectively for rednesss and ΔE, could be achieved by using this data set when developing a prediction model.

Model validation

Validation is a vital step that reveals the applicable range of a model and the limits of its performance (Gabriel 2008). This study dealt with the validation of the developed model using a set of data obtained from additional test runs, exclusive of those performed in the development of the model, as recommended by Ross (1996) and Carrasco *et al*. (2006). The experimental values of redness and ΔE were plotted against the predicted values obtained using model equations, which were in good agreement with the experimental values. Moreover, obtained predicted values with their actual values and error (%) for all 60 combinations are presented in Supporting Material 2 and error varied 0.93-32.91 % and 1.47-107.75 %, respectively for redness and ΔE. The experimental and predicted values were closely correlated as demonstrated by the regression coefficients (R^2) values of 0.87 and 0.78, respectively for redness and ΔE (Fig. 4). To confirm

the adequacy of the fitted models, the examination of residuals was also investigated. Residuals, i.e. the difference between the respective observed responses and the predicted responses, were plotted against the predicted values and observed to be scattered randomly (data not shown). Careful analysis revealed that they have no obvious pattern or unusual structure indicating that the model proposed is adequate. This suggests the variances of the original observations were constant for all responses.

Results related to the model performance are presented in Fig. 4. The applicability of the models was also quantitatively evaluated by comparing the biased and accuracy factors for each of the parameters. Overall, both AF and BF were close to 1.00 (AF for redness and ΔE =1.07 and1.21; BF for redness and ΔE=0.99 and 1.14). These values indicated that there was a good agreement between predicted and observed values. Ross *et al.* (2000) reported that predictive models should ideally have an AF=1.00 indicating a prefect model fit where the predicted and actual response values are equal. However, in the literature (Ross *et al*. 2000; Carrasco *et al*. 2006) it was reported that the AF of a model increases by 0.10-0.15 units for each predictive variable in the model. Therefore, in this study, 0.40 to 0.60 or an equivalent 40-60% variation could be expected for the AF value since four variables were included in our model. Moreover, the variation between the predicted and experimental values obtained for redness and ΔE parameters depicted by average prediction error or average mean deviation (E %) were 7.3 and 22.4%, respectively. This is within the acceptable error range as the cross validation resulted in 10.25 and 20.57 % for the same parameters. In other words, the redness and ΔE of tomato juice can be predicted within 1.4-1.6 and 3.9-6.1, respectively, for the tomato juice which had an actual redness and ΔE values of 1.5 and 5.0, respectively. Hence these instrumental value differences might not be able to be seen by observing colour with the naked eye. Consequently, based on the results obtained from

the validation experiment, the predictive performance of the established model may be considered acceptable under the experimental conditions employed. Moreover, the model was developed based on the data from nine separate experiments on fresh tomatoes of the same cultivars, from different origins and time of the year. However, the obtained results were acceptable without substantial loss of reliability, regardless of separate initial conditions. This implies that the proposed model is very likely to be an acceptable simplification of the processes occuring in reality. It also implies that the fresh juice colour of each tomato cultivar used in this experiment is consistent throughout the year and the developed model including the estimated parameters, could be comfortably applied in practice. Moreover, in this experiment a large set of data (270 data points) was used to develop two predictive models for redness and ΔE of tomato juice after blanching, unlike other modelling experiments, which might be the reason for high performance of the developed model.

It is acknowledged that in this experiment blanching was performed using a water bath which took a longer time (approximately 5 min) to reach the desired temperature in comparison to large scale conventional and novel vegetable processing industry facilities (Guns and Bayindirli 1993; Kidmose and Martens 1999; Rai *et al*. 2011). Colour values obtained after blanching might deviate from the industrially processed juice and predicted model output may not be within the expected range. However, results obtained from this experiment have proven the ability of modelling such processes in order to predict colour changes effectively after blanching or even after thermal treatment with high accuracy. Therefore, tomato juice processors can use these or similar models to support decisions on suitable cultivars and critical blanching parameters (time and temperature) to achieve the desired colour for their products without spending time and economic resources for assessing colour properties.

CONCLUSIONS

This paper presents the findings of an experimental investigation into the effect of tomato cultivar, juice pH, blanching temperature and time on colour change of tomato juice after blanching using CCD and RSM. Results reveal that maintenance of colour quality of tomato juice after blanching is mainly dependent upon the tomato cultivar and blanching temperature. The interaction and quadratic effects of cultivar, temperature and pH also had a significant influence on the maintenance of colour quality after blanching. This work clearly shows that tomato juice colour can be improved by selecting a suitable cultivar and optimising the blanching conditions. Severe thermal treatments adversely affect the red colour of tomato juice and blanching time within the studied range of 1-5 min, was not shown to significantly change colour quality. The developed models using RSM were reasonably accurate and could be used for prediction within the limits of the factors investigated. The model performance is acceptable at predicting the results of redness and ΔE, as assessed by graphical and mathematical model performance indices. The variation between the predicted and experimental values obtained for redness and ΔE were within the acceptable error range as depicted by average mean deviation (E%) of 7.3 and 22.4%, respectively. Hence one can predict the results ± 7.3 and ± 22.4% range, respectively, for redness and ΔE from the experimental or actual value. Additionally a simple and intuitive web tool (https://sites.google.com/site/tomatocolourpredict/) that utilises the developed model was created that will enable anyone to calculate the redness and total colour change of tomato juice after blanching.

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